REMARKS

The Examiner has pointed out that the sequence listing as filed with the application does not fully comply with the requirements of 37 C.F.R. §1.821-1.825 and gives as an example the DNA sequences on page 46, line 15. Applicants respectfully submit that these sequences each with 8 bases need not be identified in a sequence listing. However, in a review of the entire application, Applicants noted that one amino acid sequence was inadvertently omitted. Applicants have revised the sequence listing (see substitute sequence attached) to include this sequence. Applicants have added a sequence identifier (SEQ ID NO:27) in the specification as required. Applicants have also fixed an omission of capitalization in the sequence. Applicants believe that these additions/corrections do not constitute new matter. Please replace the originally filed sequence listing with the substitute sequence listing, which is being electronically filed as a separate document in .txt format on this date.

Claims 1-30 are pending. Group 1, claims 4 and 10 and linking claims 1-3, 5-9 and 11 have been elected. Claims 1, 5, 6, 8 and 9 have been amended. Claim 7 has been cancelled. If linked claims are allowed, Groups I- IV will be allowed. Non-elected claims are withdrawn.

Rejection under 35 U.S.C. §112

Claims 1-12 are rejected as being indefinite because of the undefined use of the term "hsiRNA". The claims have been amended accordingly relying on the definition of "hsiRNA" in the specification.

The Examiner has referred to claim 12. However, claim 12 has been withdrawn in response to the restriction request.

Rejection under 35 U.S.C. §102

The Examiner has rejected claims 1-3 as anticipated by Blaszczyk et al. and claims 1-3, 5-7 and 11 as anticipated by Sun et al. because these references describe mutated RNaseIII molecules. Both references describe the use of mutants to better understand the interaction of enzyme with substrate from a purely mechanistic perspective. Neither reference describes the potential or actual use of such mutants for gene expression which is the subject of the claimed method. This is discussed below in greater detail.

In order for a claim to be anticipated by a reference, each and every element of the claim must be taught by that reference.

The claimed invention is a new method of use of a mutant RNaseIII to generate a specified product. The application describes in detail assays for determining the activity of the mutants for producing dsRNA of a desired size range (see Figures 3-7, 10, 11) and how the activity compares with non-mutated RNaseIII activity (Figure 8), the beneficial effects on gene silencing of mutant RNAseIII (Figure 9) and the use of various mutants (for example, see Figure 12). The claimed method does not rely on the novelty of mutant RNaseIII itself but rather on the claimed use of the mutant RNAseIII.

Amended claim 1 from which the pending claims 2-4 depend states:

A method, comprising:

reacting a preparation of large dsRNA with an effective amount of a mutant RNAseIII to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are capable of being maintained in the presence of the effective amount of the mutant RNaseIII for at least 1 hour, the heterogeneous mixture being suitable for silencing gene expression (hsiRNA).

Amended claim 5 from which the pending claims 6 and 8-11 depend states:

A method, comprising:

forming a heterogeneous mixture of fragments by incubating a large double-stranded RNA (dsRNA) with a mutant RNaseIII for an effective time for cleaving, in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a fragment size of 18-30nt.

These method claims are new approaches to generating mixtures of dsRNA of specific size using effective amounts of mutant RNAs which improve the production of dsRNA of a size suitable for gene silencing from a large dsRNA.

Blaszczyk et al.

The Examiner has rejected the claimed invention as anticipated by Blaszczyk et al. because the reference describes (1) creating dsRNA

fragments of 11nt with a 3' overhang and (2) a site-directed mutation corresponding to E38 of *E. coli* RNaseIII. The Examiner has by inference suggested that this constitutes a method for generating an hsiRNA mixture suitable for gene silencing.

Applicants respectfully submit this is incorrect because the dsRNA fragments of 11nt are derived from cleavage with wild type RNaseIII. The mutation at E38 position as described by the reference is not linked to the generation of 11nt fragments.

The reference states:

> The crystal structure of the A.aelocus endonuclease domain reveals that the polar acidic side chain of E37 forms a strong hydrogen bond with the amide group of E64 which is functionally essential as suggested by the defective site directed mutant E38V of EcRNaseIII which cannot form this hydrogen bond.
>
> However, E to Q mutation at this position indeed causes no genetic defect in RNaseIII function. (see Reference at 2nd paragraph in the section entitled "Functional implications for Class 2 and 3 RNaseIII Proteins")

This description does not teach the use of the mutant discussed above or indeed any mutant for generating dsRNA of a defined size. Moreover, the reference does not describe the existence of an effective amount of the enzyme or how to determine an effective amount of such enzyme for producing a product described in the claims.

The reference therefore fails to anticipate the claimed method for reasons that include the absence of any disclosure relating to an "effective amount" of a mutant RNaseIII to achieve a certain product specified in the claimed invention.

The Examiner is therefore respectfully requested to reverse the rejection.

Sun et al.

The Examiner asserts the following:

(a) Sun et al. disclose dsRNA processing activity of a truncated

E. coli RNaseIII lacking the dsRNA binding domain.

(b) The truncated RNaseIII cleaved the 60nt transcript dsRNA.

(c) Cleavage products of RNaseIII could be visualized by phospho-imaging.

The reference describes dsRNA processing into fragments of length 47 and 13 nucleotides.

> The substrate is r1.1RNA a 60nt transcript (Fig 2A)corresponding to the bacteriophage t7 r1.1 RNAseIII processing signal. r1.1 RNA undergoes enzymatic cleavage at a a single phosphodiester within the internal loop providing products of 47 and 13 nucleotides in length. (p. 14978)

This assay in the reference does not suggest the claimed method which assays for enzymatic activity:

... to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are not substantially degraded in the presence of the effective amount of the mutant RNaseIII for at least 1 hour, the heterogeneous mixture being suitable for silencing gene expression (hsiRNA). (See amended claim 1.)

forming a heterogeneous mixture of fragments by incubating a large double-stranded RNA (dsRNA) with a mutant RNaseIII for an effective time for cleaving, in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a fragment size of 18-30nt. (See amended claim 5.)

The mutant enzyme in the reference is described as having essentially the same catalytic activity as RNAseIII under its optimal conditions (abstract, p.14979) Yet the Applicants claimed method demonstrates improved properties of mutants (see for example Figures 8 and 9) as specified by the parameters of use in the claimed method.

The requirement of an "effective amount" of a mutant RNaseIII to achieve a certain product specified in the claimed invention is not described in the reference.

For the above reasons, the Examiner is therefore respectfully requested to reverse the rejection.

Rejection under 35 U.S.C. §103

The Examiner has rejected claims 5, 8 and 9 as obvious in view of Sun et al.

(1 and 2) Determining the scope and contents of the prior art and ascertaining the differences between the prior art and the claims at issue

The scope and content of the prior art is limited to creating a mutant RNaseIII by removing the N-terminal end of the enzyme (the

RNA binding domain) and discovering that the catalytic activity is similar to the non-mutated enzyme as determined using an assay in which a 60nt substrate RNA is cleaved into a 47nt and 13nt fragment.

The Examiner identifies that the reference describes cleavage of the substrate but has ignored the requirement of (i) use of an effective amount of the mutant RNaseIII and that "(ii) at least 30% of the cleaved dsRNA has a fragment size of 18-30nt the heterogeneous mixture being suitable for silencing gene expression (hsiRNA)."

There is no suggestion or teaching based on Sun that would suggest this method of use to a person of ordinary skill in the art.

(3) Resolving the level of ordinary skill in the pertinent art

The skill in the art at the time of the reference was such that there was a desire to understand how RNaseIII cleaved dsRNA into small fragments. This was discussed in the background of the scientific reference with respect to the role of RNaseIII for cleaving mRNA (single strand RNA) and for degrading dsRNA to acid solubility. At the time of filing of the above application in 2004, Applicants who might be considered as having more than ordinary skill in the art did not know of any reference which suggested or taught the use of mutant RNaseIII for cleavage of dsRNA for gene silencing. Instead, the prevailing sentiment at the time by those of ordinary skill in the art was to use chemical synthesis to make individual short dsRNA of desired length for gene silencing.

(4) Considering objective evidence

Based on hindsight, the Examiner asserts that it would have been obvious to deduce from Sun et al. that mutant RNaseIII enzymes would cleave substrate differently from non-mutated RNaseIII. However, this is contradicted by the assertions made by Sun et al. that: (1) the mutant has the same catalytic efficiency as the wild type and (2) that *E. coli* RNaseIII could degrade dsRNA to acid solubility.

Subsequently, others attempted to control digestion dsRNA using non-mutated RNaseIII by shortening the incubation time to seconds so as to avoid degradation of dsRNA of the sort reported by Sun et al.

The present claimed invention and supporting disclosure provides an exciting new possibility for cost effective enzyme production of dsRNA mixtures for gene silencing.

Applicants respectfully request that the Examiner reverse the rejection.

CONCLUSION

Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time to file the response and have authorized that the extension fee of $555 be charged to Deposit Account No. 14-0740. Applicants authorize that any deficiencies that may be due be charged to Deposit Account No. 14-0740.

Respectfully submitted,

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